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Evaluation of the Role of Conventional and Tissue Doppler Imaging Echocardiography in Detection of Acute Cardiac Allograft Rejection in Heart Transplant Recipients

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ABSTRACT

***Background*:** Endomyocardial Biopsy (EMB) is the gold standard test for diagnosis of acute allograft cardiac rejection.
***Objectives*:** The present study aimed to assess the role of echocardiographic parameters in discriminating patients with and without evidence of acute cardiac allograft rejection.
***Materials and Methods*:** In the present cross-sectional study, using convenience sampling, 63 EMB specimens were collected from the patients who had undergone biatrial orthotropic cardiac transplantation. The mean age of the recipients and donors was 30.46 ± 9.49 and 24.55 ± 7.64 years, respectively. There were 51(81%) male recipients and 39(62%) male donors. Echocardiographic examination was performed within the 24 hours of EMB. The data were entered into the SPSS statistical software, version 19 and were analyzed by chi-square test, student's t-test, and one-way ANOVA as appropriated. All the data were two-tailed and $P < 0.05$ was considered to be statistically significant.
***Results*:** Among the 63 EMB specimens evaluated in the present study, mild and moderate acute rejections were seen in 19(30%) and 5(8%) cases, respectively. On Doppler examination, the three groups (without rejection, with mild rejection, and with moderate acute rejection) were significantly different only regarding trans-tricuspid E wave ($P = 0.040$). Pulsed-wave Tissue Doppler Imaging (TDI) also revealed a significant difference between the patients with and without allograft rejection regarding early diastolic tricuspid and mitral annular motion velocities ($P = 0.005$ and $P = 0.02$, respectively).
***Conclusions*:** It seems that echocardiographic parameters, including TDI, might be adjunct to, rather than substitution for, EMB findings for early diagnosis of acute allograft rejection.

►*Implication for health policy/practice/research/medical education:*
Cardiac transplantation is a life-saving treatment option for patients with end-stage heart failure. Despite advanced immunosuppressive regimes, acute cardiac allograft rejection is still the major cause of morbidity and mortality in the first year post-transplantation. Identification of patients at an earlier stage would prevent progression to more severe disease and ultimately reduces the risk of long-term complications. In the present study, we assessed the clinical applicability of tissue Doppler imaging techniques in the early diagnosis of acute cardiac allograft rejection.

1. Background

Cardiac transplantation is considered to be a life-saving treatment option for patients with end-stage heart failure. However, rejection occurs in most patients. Cardiac allograft rejection is classified into three major categories, namely hyperacute, acute, and chronic. Hyperacute rejection occurs within minutes to hours after transplantation and

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is caused by preexisting antibodies to allogenic antigens. Acute rejection is classified into acute cellular rejection (or cell-mediated rejection) and acute humoral rejection (or antibody-mediated rejection). Acute cellular rejection most commonly occurs within the first weeks to several years after transplantation. Finally, chronic rejection occurs within months to years after transplantation. Despite advanced immunosuppressive regimes, Acute Cardiac Allograft Rejection (ACAR), with an incidence rate of over 40%, is still the major cause of morbidity and mortality in the first year post-transplantation (1). Clinical features of allograft rejection are unreliable and most patients often remain asymptomatic until hemodynamic complications ensue (2). Routine screening is therefore performed at fixed schedules to detect allograft rejection. Identification of patients at an earlier stage followed by early immunosuppressive therapy would prevent progression to more severe disease and ultimately reduces the risk of long-term complications. Right ventricular Endomyocardial Biopsy (EMB) and histological evaluation of the myocardial tissue constitute the gold standard surveillance tool after cardiac transplantation (3, 4). However, this procedure is invasive, expensive, and time-consuming. Moreover, due to sampling errors related to the patchy nature of allograft rejection as well as inter-observer variability in the interpretation of histological findings, 'biopsy-negative' rejection occurs in up to 20% of patients (5). Uncommon but potentially serious complications, such as tricuspid valve insufficiency, coronary artery-right ventricular fistula, carotid artery puncture, arrhythmia, and prolonged bleeding, may also occur (4, 6-9).

Extensive studies have attempted to develop a non-invasive diagnostic tool to screen patients at risk of acute allograft rejection. An accurate and non-invasive method with desirable patient comfort, risk, convenience, speed, and cost can eliminate or decrease the need for serial EMB. However, data are still insufficient and conflicting regarding the role of echocardiography in detection of ACAR.

2. Objectives

The present study aims to evaluate the clinical applicability of systolic and diastolic echocardiographic indices, particularly Tissue Doppler Imaging (TDI) techniques, in early diagnosis of ACAR.

3. Materials and Methods

After gaining the approval of the institutional Ethics Committee and obtaining written informed consents, using convenience sampling, 63 EMB specimens were obtained from heart transplant recipients to be included in the present cross-sectional study. All the required data were collected via a paper-based questionnaire completed by one of the investigators (MA). Right ventricular myocardial biopsy using International Society of Heart and Lung Transplantation (ISHLT) 2005 grading system was used as the gold standard test for diagnosis of acute myocardial rejection (10).

3.1. Patients

All the patients undergoing orthotopic cardiac transplantation at Rajaei Cardiovascular Medical and Research Center, Imam Khomeini hospital, and Shariati Hospital, Tehran, Iran during 2008 - 2011 were consecutively enrolled into the present study. The patients who had post-transplant valvular prostheses, re-transplantation, and insufficient imaging quality for analysis were excluded from the study.

3.2. Cardiac Transplantation

All the heart transplantations were performed by a single team with one surgeon through Biatrial (BA) orthotopic heart transplant surgical technique. The BA technique was performed as described by Lower and Shumway (11). Donor heart preservation consisted of hypothermic cardioplegic arrest and storage at 4°C after perfusion with Stanford solution.

3.3. Cardiac Catheterization and Endomyocardial Biopsy

As per institutional policy, acute rejection is monitored by serial myocardial biopsies started from day 14 post-transplantation and performed weekly during the first month, every 2 weeks in the second and third months, monthly until the 6th month, every 3 months until the end of the second year, and at 6-month intervals thereafter. A myocardial biopsy was also obtained whenever clinical suspicion of transplant rejection was present.

Right-side catheterization was performed using standard internal jugular venous approach. After inserting a 7-9F sheath in the right internal jugular vein, the bioptome was guided through the tricuspid valve into the right ventricle. Four to eight samples were taken at each catheterization from the mid-portion of the interventricular septum. Afterwards, the samples were immediately fixed in formaldehyde and were sent to the pathology laboratory.

3.4. Pathological Evaluations

The specimens were embedded in paraffin and hematoxylin-eosin dye was used to examine the tissue samples. All the biopsies were read by a single experienced pathologist who was blinded to the cardiac echocardiographic findings. Cellular rejection was graded according to the revised 2005 ISHLT grading system as follows: grade 0R biopsies were considered as negative for cellular rejection, grade 1R biopsies as mild cellular rejection, grade 2R as moderate cellular rejection, and grade 3R biopsies as severe cellular rejection (10).

3.5. Echocardiography

A conventional transthoracic echocardiographic examination together with acquisition of myocardial velocity imaging was performed on each patient within 24 hours of the biopsy procedure using a Vivid 7 ultrasound system (GE Medical System, Horton, Norway) and a 1.7/3.4 MHz transducer. All the echocardiographic studies were performed by a single echocardiographist who was totally blinded to the pathology results.

3.6. Standard Echocardiography

Left Ventricular (LV) M-mode measurements included septal and posterior wall thickness at end diastole,

interventricular septum, and right ventricular free wall all measured from an apical four-chamber view. LV volume and Ejection Fraction (EF) were measured using Simpson's method through apical two- and four-chamber views. In addition, left and right ventricular fillings were assessed by measuring inflow at the tips of the leaflets of the mitral and tricuspid valves using pulsed Doppler. The following parameters were measured: early diastolic peak flow velocity (E, cm/s), late diastolic velocity (A, cm/s), and time to E-wave (measured from the beginning of the QRS to the peak E wave, msec). The Myocardial Performance Index (MPI), also known as Tei index, was calculated using the following formula:

$$MPI = \frac{IVCT + IVRT}{ET}$$

(IVCT, isovolumic contraction time; IVRT, isovolumic relaxation time; ET, ejection time).

3.7. Tissue Doppler Myocardial Imaging

Longitudinal myocardial velocity profiles were obtained from the 4-chamber left apical view. Peak systolic (Sm), early diastolic (Em), and late diastolic (Am) velocities were measured off-line at the base and middle segments of the septum, LV lateral wall, and right ventricular free (lateral) wall by Pulsed-Wave Tissue Velocity Imaging (PW-TVI) and Color-Coded Tissue Velocity Imaging (CD-TVI).

3.8. Immunosuppressive Regimen

All the patients received Intravenous (IV) administration of l000 mg methylprednisolone during the operation. Methylprednisolone (250 mg per day) was also given intravenously in two divided doses in the first 2 days after the operation followed by 1 mg. kg-1 oral prednisolone thereafter. Prednisolone was tapered by 5 mg until reaching the dose of 15 mg per day, which was continued for at least one year later. Besides, 1000 mg mycophenolate mofetil (CellCept®) was administered prior to the operation and was continued as 1000 mg twice a day lifelong. Moreover, 100 mg rabbit Anti-Thymocyte Globulin (ATG) per day was infused over 4-8 hours during the first 3 days post operation. Cyclosporine was also administered based on the serum level beginning from the second day post-operation and continuing lifelong.

3.9. Data Analysis

All the analyses were performed by Statistical Package for Social Sciences software, version 19 (SPSS Inc, Chicago, IL). All the values were expressed as mean ± SD for continuous variables and number (percentage) for categorical ones. Normality was evaluated for each variable on the basis of normal distribution plots and histograms and by Kolmogorov-Smirnov test. The two groups were compared regarding clinical characteristics and echocardiographic indices using chi-square test for categorical variables and student's t-test, Mann-Whitney test, Kruskal-Wallis test, and one-way ANOVA for quantitative variables as appropriated. All the data were two-tailed and $P < 0.05$ was considered to be statistically significant.

4. Results

4.1. Patients' Characteristics and Clinical Data

In total, 63 specimens were presented. The mean age of the recipients and donors was 30.46 ± 9.49 and 24.55 ± 7.64 years, respectively. Besides, the mean follow-up period after orthotopic cardiac transplantation was 68.11 ± 62.15 weeks, ranging from 2 to 265 weeks.

Endomyocardial histopathology revealed ISHLT rejection grade 0 (no rejection) in 39 (61.9%), grade 1R (mild rejection) in 19 (31.16%), and grade 2R (moderate rejection) in 5 (7.93%) samples. No cases of ISHLT grade 3R were seen during the study period. Demographic characteristics of the group without ACAR (N = 39), the group with mild ACAR (N = 19), and the group with moderate ACAR (N = 5) have been presented in Table 1.

4.2. Conventional Echocardiographic Data

The study groups' conventional echocardiographic parameters based on the ISHLT grades have been show in Table 2. Accordingly, no significant differences were observed among the study groups in terms of conventional echocardiographic parameters.

Table 1. Demographic Characteristics of the Recipients and Donors

	ISHLT Grading 2005			P value
	No rejection (Grade 0) N = 39	Mild rejection (Grade 1R) N = 19	Moderate rejection (Grade 2R) N = 5	
Recipient's age, yrs	29.6 ± 18.26	30.10 ± 11.57	38.40 ± 7.70	0.132
Recipient's sex, n (%)				
Male	32 (63)	14 (27)	5 (10)	0.273
Female	7 (59)	5 (41)	(0)	
Height, cm	168.84 ± 9.04	167.36 ± 13.53	172.60 ± 10.01	0.692
Weight, kg	68.98 ± 18.66	64.94 ± 16.93	79.20 ± 32.07	0.440
BMI, kg/m2	23.84 ± 5.05	23.10 ± 5.57	26.69 ± 4.96	0.401
BSA, m2	1.77 ± 0.25	1.72 ± 0.26	1.97 ± 0.29	0.312
Donor's age, yrs	24.88 ± 7.36	22.73 ± 7.82	29.00 ± 6.86	0.155
Donor's sex, n (%)				
Male	25 (64)	10 (26)	4 (10)	0.075
Female	14 (59)	9 (37.5)	1 (4.5)	
Time from HTx, weeks	32.5 (8.5 - 86.0)	42.0 (24.5 - 111.0)	56.0 (17.0 - 137.5)	0.385

Abbreviations: BMI, body mass index; BSA, body surface area; HTx, heart transplantation. Data are presented as mean ± SD or median (interquartile range) and n (%).

Table 2. Conventional Echocardiographic Characteristics

	ISHLT Grading 2005			P value
	No rejection (Grade 0) N = 39	Mild rejection (Grade 1R) N = 19	Moderate rejection (Grade 2R) N = 5	
PWT (cm)	0.95 ± 0.12	0.94 ± 0.15	1.00 ± 0.18	0.751
IVST (cm)	0.96 ± 0.10	0.94 ± 0.12	0.94 ± 0.13	0.721
LVEDD (cm)	4.47 ± 0.54	4.38 ± 0.52	4.42 ± 0.46	0.871
LVESD (cm)	2.93 ± 0.62	2.73 ± 0.45	2.98 ± 0.123	0.392
LVEDV (mL)	75.21 ± 18.81	69.18 ± 17.79	66.84 ± 11.87	0.386
LVESV (mL)	31.45 ± 12.18	27.43 ± 7.44	28.34 ± 5.02	0.552
RAEDV (mL)	29.82 ± 13.17	27.94 ± 9.08	27.60 ± 14.67	0.988
RAESV (mL)	41.65 ± 16.86	41.08 ± 10.22	40.70 ± 23.11	0.966
LAEDV (mL)	38.96 ± 20.33	48.24 ± 29.15	50.25 ± 24.95	0.425
LAEDA (cm2)	15.59 ± 5.46	17.64 ± 7.36	18.132 ± 5.66	0.424
LV mass (gr)	144.39 ± 33.49	139.65 ± 43.68	146.16 ± 44.79	0.802
LVEF (%)	58.05 ± 8.85	59.57 ± 6.06	74.84 ± 4.14	0.448
RVFAS (%)	43.64 ± 7.65	44.94 ± 6.38	45.40 ± 6.26	0.976
RWMA Score Index	1.13 ± 0.27	1.07 ± 0.09	1.08 ± 0.07	0.769
TRG (mmHg)	27.5 ± 7.47	22.88 ± 5.67	23.60 ± 5.94	0.191
PAP (mmHg)	33.70 ± 8.15	28.82 ± 5.57	32.00 ± 7.61	0.181
TAPSE (mm)	17.11 ± 3.098	17.30 ± 3.27	17.40 ± 5.36	0.931
PE, n (%)				
No	30 (76.9%)	14 (73.3%)	4 (80%)	
Small	5 (12.8%)	5 (26.3%)	0 (0%)	
Moderate	5 (5.1%)	0 (0%)	1 (20%)	0.723
Large	2 (5.1%)	0 (0%)	0 (0%)	
Total	39 (100%)	19 (100%)	5 (100%)	

Abbreviations: IVST, interventricular septum thickness; LAEDA, left atrialo end diastolic area; LAEDV, left atrial end diastolic volume; LV, left ventricle; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end diastolic dimension; LVEDV, left ventricular end diastolic volume; LVESD, left ventricular end systolic dimension; LVESV, left ventricular end systolic volume; PAP, pulmonary artery pressure; PE, pericardial effusion; PWT, posterior wall thickness; RAEDV, right atrial end diastolic volume; RAESV, right atrial end systolic volume; RVFAS, right ventricular fractional area shortening; RWMA, regional wall motion abnormality; TAPSE, tricuspid annular plane systolic excursion; TRG, tricuspid regurgitation gradient
Data are presented as mean ± SD and n (%).

4.3. Doppler Echocardiographic Indices

On Doppler examination, there were no significant differences among the patients with different ISHLT grades concerning mitral valve inflow and left and right ventricular MPIs (Table 3). The only parameter which was significantly different among the patients with different ISHLT grades was tricuspid E-wave velocity (P = 0.040). This measure of diastolic function was slightly lower in the patients with moderate ACAR compared to those with no or mild ACAR.

4.4. Mitral and Tricuspid Annular Motion Velocity Data

Pulsed-wave TDI revealed significant differences among the patients with no, mild, and moderate ACAR with regard to tricuspid annular early diastolic velocity (Em) (P = 0.019,

Table 3. Transmitral and Trans-Tricuspid Doppler Data

	ISHLT Grading 2005			P value
	No rejection (Grade 0) N = 39	Mild rejection (Grade 1R) N = 19	Moderate rejection (Grade 2R) N = 5	
Transmitral Doppler data				
E-wave (m/s)	0.79 ± 0.19	0.83 ± 0.19	0.88 ± 0.21	0.625
A-wave (m/s)	0.45 ± 0.11	0.145 ± 0.09	0.45 ± 0.21	0.483
E to A ratio	1.83 ± 0.53	1.85 ± 0.35	2.13 ± 0.58	0.487
Time to E-wave (msec)	449.94 ± 37.33	445.72 ± 29.88	438.60 ± 51.88	0.681
Transtricuspid Doppler data				
E-wave (m/s)	0.60 ± 0.18	0.70 ± 0.18	0.52 ± 0.10	0.040
A-wave (m/s)	0.49 ± 0.13	0.53 ± 0.15	0.55 ± 0.15	0.539
E to A ratio	1.28 ± 0.37	1.39 ± 0.36	0.95 ± 0.37	0.192
Time to E-wave (msec)	448.82 ± 33.82	438.52 ± 35.25	447.20 ± 66.57	0.505
Myocardial performance index				
LV-MPI	0.41 ± 0.13	0.42 ± 0.11	0.36 ± 0.05	0.536
RV-MPI	0.38 ± 0.10	0.36 ± 0.11	0.38 ± 0.08	0.805

Abbreviations: LV, left ventricle; MPI, myocardial performance index; RV, right ventricle.
Data are presented as mean ± SD.

Table 4). Moreover, significant differences were observed between the patients with and without allograft rejection concerning early diastolic tricuspid and mitral annular motion velocities (P = 0.005 and P = 0.024, respectively).

4.5. Color-Coded Tissue Doppler Imaging Data

Color-coded TDI study demonstrated significant differences among the patients with no, mild, and moderate ACAR regarding systolic velocity (Sm) and acceleration rate of Sm (Sm-AR) of the basal segment of the right ventricular free wall (P = 0.011 and P = 0.017, respectively) (Table 5). Moreover, Em, Sm, and Sm-AR of the basal segment of the right ventricular lateral (free) wall were significantly higher in the patients with ACAR (grade ≥ 1R) compared to those without ACAR (P = 0.004, P = 0.021, and P = 0.007, respectively). The patients with various ACAR groups were also significantly different with respect to Em of the middle segment of the LV lateral wall (P = 0.017).

5. Discussion

Serial EMB is still the gold standard test for detection of cardiac allograft rejection. However, it is not convenient and has potential limitations and complications (3, 4). As a result, there has been increasing interest in defining alternative non-invasive diagnostic approaches.

Some investigators applied cardiac troponin (cTn) as an indicator of cardiac allograft rejection and almost failed to show any significant relationship between cTnT or cTnI levels and rejection grade (12-16). Mullen et al. also observed that cTn could not accurately show acute cardiac rejection over a mean follow-up of 129 ± 9 days (14). Several non-invasive techniques have also been evaluated in this regard, including radionuclide imaging, magnetic resonance imaging, intracardiac electrogram recording, and multiparametric immune monitoring (17-20). However, none of these non-invasive imaging techniques were found sufficiently reliable to replace EMB. Echocardiography has been in extensive use by cardiologists in the clinical surveillance of heart transplant recipients and almost all known echocardiographic studies have been on trials to determine whether they can predict acute allograft rejection. The first use of echocardiography to diagnose acute rejection is credited to Schroeder who demonstrated that acute rejection was associated with an increase in posterior LV wall thickness (21). This was further shown in other trials using M-mode echocardiography and measuring ventricular mass (22-25). Cardiac magnetic resonance imaging has also shown increased thickness of the LV septum, apex, and lateral walls in moderate transplant rejection (24).

Assuming a restrictive physiology due to myocardial infiltration, studies then applied echocardiographic indices to show whether restrictive physiology could predict allograft rejection. Putting all the data together, it seems that an apparent restrictive physiology is quite specific, but lacks sensitivity to moderate degree of allograft rejection since many recipients without rejection also showed restrictive physiology (26-30). However, we did not observe any significant restrictive physiology in our rejection cases. In addition to all the technical issues in measuring diastolic indices, we think early detection of cellular rejection on a routine EMB schedule should prevent the allograft from going into a severe restrictive state and, consequently, the related echocardiography indices should not be very useful.

In agreement with the previous studies, we also could not demonstrate any role for echocardiographic markers of systolic dysfunction (31). Although Paulsen et al. demonstrated that LVEF declined in patients with acute rejection (32), it is worth noting that decrease of EF in patients with less severe allograft rejection may be subtle enough to be neglected by echocardiographic examination.

MPI, combining systolic and diastolic function, is calculated as the sum of the Isovolumic Contraction Time (IVCT) and Isovolumic Relaxation Time (IVRT) divided by the ejection time. Burgess et al. (33) found an increase in IVCT and a decrease in IVRT during rejection with no significant change in the MPI. Vivekananthan et al. also showed that an MPI increase of ≥ 20% from baseline had 90% sensitivity and 90% specificity in detecting high-grade cardiac allograft rejection (34). This finding was not confirmed in other studies (35, 36). Similarly, we found no benefits in applying right and left ventricular myocardial

Table 4. Pulse-Wave tissue Doppler Imaging Mitral and Tricuspid Annular Motion Velocity Data

		ISHLT Grading 2005			P value
		No rejection (Grade 0) N = 39	Mild rejection (Grade 1R) N = 19	Moderate rejection (Grade 2R) N = 5	
RV	Sm (m/s)	0.84 ± 0.25	0.95 ± 0.16	0.91 ± 0.22	0.242
	Em (m/s)	0.77 ± 0.32	0.96 ± 0.26	1.02 ± 0.32	0.019
	Am (m/s)	0.86 ± 0.35	0.79 ± 0.30	1.06 ± 0.54	0.643
	E/Em ratio	8.93 ± 4.32	7.83 ± 2.95	5.60 ± 2.50	0.205
Septum	Sm (m/s)	0.70 ± 0.18	0.73 ± 0.10	0.72 ± 0.16	0.784
	Em (m/s)	0.82 ± 0.21	0.88 ± 0.21	0.80 ± 0.19	0.464
	Am (m/s)	0.59 ± 0.18	0.53 ± 0.15	0.58 ± 0.16	0.556
	E/Em ratio	10.20 ± 3.42	10.11 ± 3.38	11.75 ± 5.67	0.953
LV lateral	Sm (m/s)	0.82 ± 0.26	0.89 ± 0.15	0.76 ± 0.13	0.143
	Em (m/s)	1.20 ± 0.32	1.48 ± 0.36	1.33 ± 0.36	0.061
	Am (m/s)	0.53 ± 0.24	0.53 ± 0.17	0.47 ± 0.14	0.911
	E/Em ratio	7.05 ± 2.75	6.12 ± 1.89	7.00 ± 2.64	0.560

Abbreviations: Am, late diastolic velocity; Em, early diastolic velocity; LV, left ventricle; RV, right ventricle; Sm, systolic velocity. Data are presented as mean ± SD.

Table 5. Color-Coded Tissue Doppler Imaging Data

		ISHLT Grading 2005			P value
		No rejection (Grade 0) N = 39	Mild rejection (Grade 1R) N = 19	Moderate rejection (Grade 2R) N = 5	
RV-base	Sm (cm/sec)	5.88 ± 2.37	8.13 ± 1.53	7.16 ± 2.58	0.011
	Em (cm/sec)	5.75 ± 2.970	7.54 ± 1.98	7.33 ± 3.16	0.084
	Am (cm/sec)	4.67 ± 2.62	4.51 ± 2.27	7.40 ± 4.04	0.320
	Time of Sm (sec)	153.45 ± 31.48	154.06 ± 23.12	149.60 ± 51.23	0.831
	Time to Em (msec)	456.34 ± 45.08	446.13 ± 31.61	438.40 ± 55.11	0.489
	Sm-AR (cm/sec2)	39.50 ± 18.44	54.11 ± 14.00	53.29 ± 31.000	0.017
	E-Em (sec)	28.77 ± 24.13	23.80 ± 24.01	19.60 ± 23.26	0.296
RV-middle	Sm (cm/sec)	3.75 ± 1.70	4.74 ± 1.59	4.42 ± 2.95	0.232
	Em (cm/sec)	2.96 ± 1.77	3.92 ± 1.73	3.89 ± 1.08	0.149
	Am (cm/sec)	2.38 ± 1.55	2.43 ± 1.71	5.43 ± 2.95	0.150
	Time of Sm (sec)	151.26 ± 34.88	152.66 ± 25.16	148.50 ± 61.90	0.861
	Time to Em (msec)	466.48 ± 45.21	453.85 ± 45.86	417.25 ± 53.84	0.236
	Sm-AR (cm/sec2)	25.47 ± 11.88	31.72 ± 14.21	37.38 ± 36.74	0.416
	E-Em (sec)	37.56 ± 26.82	36.14 ± 19.80	40.50 ± 31.83	0.958
Septum-base	Sm (cm/sec)	4.77 ± 1.40	5.10 ± 1.01	5.21 ± 0.50	0.321
	Em (cm/sec)	6.26 ± 2.15	6.41 ± 1.64	6.79 ± 2.10	0.990
	Am (cm/sec)	3.16 ± 1.36	3.10 ± 1.15	3.52 ± 1.82	0.800
	Time of Sm (sec)	140.69 ± 28.24	142.56 ± 13.75	128.80 ± 28.18	0.624
	Time to Em (msec)	458 ± 49.33	461.50 ± 33.91	45.40 ± 71.69	0.731
	Sm-AR (cm/sec2)	35.13 ± 11.14	36.10 ± 7.70	42.08 ± 9.68	0.396
	E-Em (sec)	29.30 ± 25.74	25.00 ± 13.77	20.00 ± 16.38	0.446
Septum-middle	Sm (cm/sec)	2.41 ± 1.12	2.59 ± 0.75	2.75 ± 0.95	0.499
	Em (cm/sec)	4.01 ± 1.55	4.25 ± 2.48	3.89 ± 2.99	0.898
	Am (cm/sec)	1.56 ± 1.09	1.67 ± 0.89	1.48 ± 0.50	0.798
	Time of Sm (sec)	143.45 ± 34.31	137.87 ± 17.53	115.80 ± 33.72	0.184
	Time to Em (msec)	470.21 ± 53.00	466.62 ± 30.59	447.60 ± 77.46	0.534
	Sm-AR (cm/sec2)	17.80 ± 8.40	19.19 ± 6.64	23.87 ± 6.62	0.259
	E-Em (sec)	35.63 ± 25.51	26.46 ± 13.55	32.20 ± 16.60	0.518
LV lateral-base	Sm (cm/sec)	6.39 ± 2.16	6.01 ± 1.91	5.45 ± 1.44	0.527
	Em (cm/sec)	9.58 ± 2.52	10.40 ± 2.68	8.48 ± 4.61	0.636
	Am (cm/sec)	2.93 ± 2.27	2.06 ± 1.61	2.08 ± 1.45	0.545
	Time of Sm (sec)	150.77 ± 38.31	165.33 ± 46.55	165.80 ± 46.26	0.492
	Time to Em (msec)	460.68 ± 32.48	451.53 ± 32.13	445.20 ± 63.93	0.442
	Sm-AR (cm/sec2)	45.98 ± 22.32	39.65 ± 16.24	36.34 ± 17.42	0.610
	E-Em (sec)	20.51 ± 16.44	19.28 ± 12.14	23.00 ± 15.48	1
LV lateral-middle	Sm (cm/sec)	4.41 ± 2.07	4.71 ± 1.77	3.91 ± 0.88	0.781
	Em (cm/sec)	5.60 ± 2.12	7.61 ± 2.32	5.28 ± 2.80	0.016
	Am (cm/sec)	2.02 ± 2.13	2.09 ± 1.48	1.65 ± 1.27	0.849
	Time of Sm (sec)	154.08 ± 45.16	173.06 ± 49.69	168.80 ± 55.69	0.247
	Time to Em (msec)	462.45 ± 39.65	450.13 ± 33.95	438.40 ± 65.39	0.324
	Sm-AR (cm/sec2)	32.57 ± 39.65	30.18 ± 16.29	25.92 ± 12.38	0.860
	E-Em (sec)	28.51 ± 21.66	20.00 ± 17.20	19.80 ± 12.26	0.192

Abbreviations: Am, late diastolic velocity; AR, acceleration rate; Em, early diastolic velocity; LV, left ventricle; RV, right ventricle; Sm, systolic velocity.
Data are presented as mean ± SD

performance to predict allograft rejection.

TDI-derived indices have been shown to be useful in predicting subtle cardiac injuries when conventional indices of LV function are still normal. In a study conducted on nearly 400 biopsies using PW-TDI, significant allograft rejection (≥ grade 2) was shown to be associated with a significantly reduced peak early diastolic velocity (Em,) and a significantly prolonged early diastolic time (TEm) of the basal LV inferolateral wall (37). Three smaller studies have also demonstrated that Em significantly reduced in allograft rejection (23, 38, 39). On the other hand, other studies on 1500 biopsies in nearly 450 patients reported no association between acute allograft rejection and Em (40-46). Our study found that both tricuspid and mitral annular early (Em), but not late (Am), diastolic velocities were significantly different in the patients with acute allograft rejection. Stengel et al. (45) showed that Am and mitral annular systolic contraction velocity were higher in patients with higher degrees of rejection. Likewise, Kato et al. indicated that neither conventional echocardiographic indices nor Em or the E/Em mitral flow ratio could differentiate patients with and without allograft rejection (41).

In a study by Mankad et al., patients with biopsy-proven rejections had lower tissue Doppler posterior wall peak

systolic and diastolic velocity gradients compared to those without rejection (38). They believed that although TDI could not differentiate rejection from other causes of low velocity values, high TDI velocity values could rule out rejection.

The present study results demonstrated higher early diastolic velocity (Em) of the basal segment of the right ventricular free wall in the patients with cardiac rejection. The results also showed that Em of the middle segment of the LV lateral wall was significantly different among various ACAR groups.

The major limitation of this study was its small sample size. Thus, the negative results obtained in this study might actually be a type II error. Another limitation of this study was that we did not assess the presence of antibody-mediated rejection, which might have altered our results. The patchy and heterogeneous nature of the rejection process as well as the potential errors occurred during the biopsy might also have influenced our results.

Overall, the results of our study were not sufficient to suggest the use of echocardiographic parameters, even TDI-derived indices, to replace surveillance EMB for detection of acute allograft rejection. Echocardiography indices are certainly helpful in allograft rejection patients, but the results should be always interpreted along with clinical and biopsy findings. Nonetheless, due to the potential benefits that non-invasive assessment of rejection could offer in heart transplant recipients, search for sensitive and specific non-invasive tools that can predict acute allograft rejection warrants to be continued by further prospective studies.

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Authors' Contribution

Zahra Ojaghi Haghighi, Sepideh Taghav, Mahsa Abdollahi, Nasim Naderi, and Kambiz Mozaffari contributed to study concept and design and data collection. Ahmad Amin and Mitra Chitsazan contributed to analysis and interpretation of the data, drafting and critical revision of the manuscript, and approval of the article.

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